NASA microgravity research highlights

Caught on film: Nucleating crystals make their debut

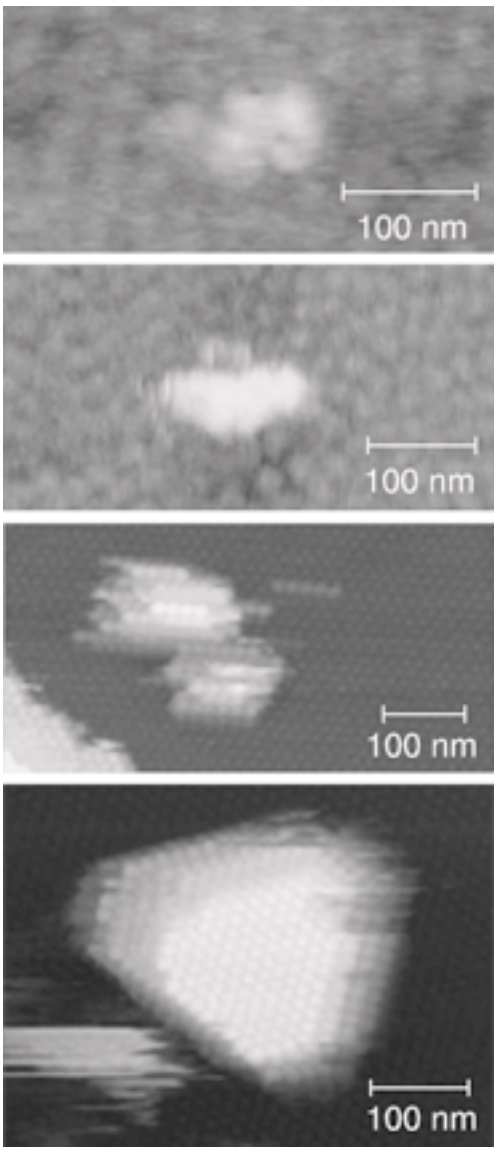
Reprinted from the Fall 2000 issue of *Microgravity News*

What if you could witness the beginning of a process that has long been theorized but never seen? You might understand that process better, finding ways to keep it in check or to enhance it, and you might be surprised by what you saw. That's just what biotechnology Principal Investigator Peter Vekilov, of the University of Alabama in Huntsville (UAH), and his team of researchers discovered. They have established a method that lets them visualize the very beginning stages of the growth of protein crystals at the molecular level.

Vekilov's team at UAH studies protein solutions as they change phases from liquids to crystalline solids. They want to know if the molecules in the solution interact with one another, and if so, how, from the perspectives of thermodynamics and kinetics. They want to understand which forces — electrical, electrostatic, hydrodynamic, or other kinds of forces — are responsible for the interactions. They also study nucleation, the beginning stage of crystallization. This process is important to understand because it sets the stage for crystal growth in all kinds of solutions and liquid melts that are important in such diverse fields as agriculture, medicine, and the fabrication of metal components. Nucleation can determine the rate of crystal growth, the number of crystals that will be formed, and the quality and size of the crystals.

The formation of a crystal, Vekilov explains, starts when a small number of molecules bump into each other, and their energy is such that it is more favorable for them to come together than to remain apart. These molecules create a new surface in the protein solution, and in order to continue growing, that tiny surface requires energy. Energy input or energy loss determines whether the molecules continue to attract other molecules or whether the small structure breaks down into its constituent parts. Thus, nucleation only occurs when the surface energy barrier is successfully overcome, allowing more molecules to join the aggregate.

Observing the beginning of crystal growth has been hampered by the small size of the molecules, the speed at which nucleation occurs, and the ability to pinpoint just where in a volume of liquid the nucleation events are taking place. Fortunately for Vekilov and his team, the molecules of the protein that they study are relatively large, at approximately 130 angstroms in length (compared to a water molecule, which is only about 2 angstroms), and molecules enter and leave the nucleus every two to three seconds, which is within the capability of an atomic force microscope. Locating the site of nucleation within a volume of liquid has proven to be the greatest difficulty. Vekilov's team solved this problem by waiting for the nuclei, formed in the bulk of the liquid, to attach to the bottom of the container holding the protein solution. They discovered that plain, uncoated glass, which is not typically used for protein crystal growth, provided the best substrate for their observations because the nuclei stick to it and can be filmed.



Watching molecules of the iron-storing protein apoferritin come together to form a nucleus reveals some interesting behavior. In this series of images, researchers observed clusters of four molecules at the corners of a diamond (top). As more molecules attach to the cluster, they arrange themselves into rods (second from top), and a raft-like configuration of molecules forms the critical nucleus (third from top), suggesting that crystal growth is much slower than it could be were the molecules arranged in a more compact formation. In the final image, a crystallite consisting of three layers containing approximately 60 to 70 molecules each is formed. Atomic force microscopy made visualizing the process of nucleation possible for the first time.

Using atomic force microscopy, a skilled operator like Sun-Tung Yau, a researcher in Vekilov's laboratory, can create subatomic image sequences showing the dynamic behavior of individual molecules as they attach and detach from a crystal or crystalline nucleus. Vekilov explains that the imaging process of the atomic force microscope is a little like being able to see and count your ribs while you trace your finger along your chest wall. The microscope has a very sharp tip and a spring mechanism that acts as a cantilever. It works by measuring how much force is pressing against the tip as the tip is scanned over the surface of the container holding the nuclei. The deflection of the tip allows the nuclei to be "seen" by the microscope. Using this method, Vekilov and his team have filmed the joining of individual apoferritin molecules (proteins that store iron in the body) into a nucleus 20 to 50 molecules in size. In some 600 images, the dynamic behavior of the growing crystal was revealed.

What Vekilov and his team saw using the atomic force microscopy technique surprised them. "You would think that when a crystal grows, it is like a car going forward on a highway," he says. "There is a driving force, and it should go, but it doesn't. At the molecular level, a molecule may enter the crystal, and then it may detach from the crystal. It's not a linear process. It actually goes back and forth all the time. It is just that the attachment events occur more often than the detachment events. You can theorize that this is what is happening, but we saw it," Vekilov explains excitedly. Prior to this discovery, scientists gauged nucleation rates indirectly by counting crystals once they had grown to macroscopic size. With this discovery, it's now possible to directly measure nucle-

ation rates in the very smallest of aggregates.

The surface and shape of the nuclei Vekilov's team observed in the apoferritin solution provided another surprising discovery. "Since the surface energy of the nuclei determines the nucleation process, you can imagine that nuclei of various shapes will have different surface energy factors," he explains. "A square nucleus will be different from a round nucleus, for example, a rough surface, different from a smooth one. The surprising thing is that those nuclei we observed were actually shaped like a raft." The quasiplanar shape of the nuclei is unusual, Vekilov explains, because it has a large surface area compared to a more compact shape, such as a sphere. The greater the surface area, the more energy that is required for crystallization. Studies of the shape of the nuclei showed a strong correlation with studies of the kinetics of crystal growth. "This raft-like shape says that nucleation is actually much slower than what it could be were those nuclei spherical," Vekilov concludes.

Although the apoferritin molecules revealed these surprising results, Vekilov does not expect all proteins to show such unusual behavior. It is the ability to see this process at all that he finds exciting. Observing the beginning stages of crystal growth may lead researchers to a better understanding of the rest of the growth process, including the effects of gravity and microgravity on crystal growth and why some crystals grow better in space than others. Prior to his team's discoveries, says Vekilov, researchers may indeed have seen the nucleus, but it was difficult to show that that was what it was. "We were able to see the nuclei and show that they are what we think they are."

Just as observing the nuclei has been difficult for researchers, studying the kinetics of the nucleation process has also required a great deal of skill and patience, explains Vekilov. Dust particles in the protein solution can trigger nucleation by acting as a surface for molecules to attach to, thereby disrupting the natural forces that bring molecules into contact with one another. Researchers are only interested in natural nucleation and must factor out these dust particle events. In studies of lysozyme, which comprises the bulk of hen egg whites, Vekilov and his team developed a method to distinguish between this so-called heterogenous nucleation, triggered by dust particles suspended in the solution, and homogeneous nucleation, when the process begins on its own.

What became clear from these studies, says Vekilov, is a rationale for the control of the nucleation rate of protein crystals. Additions of glycerol to the protein solution work to suppress nucleation, while adding polyethylene glycol to the protein solution works to enhance the nucleation process. Neither of these substances interact with the protein molecules, making them very useful for controlling nucleation. Vekilov explains that if one could control the nucleation process, one could potentially affect the pathological growth of protein crystals that results in the formation of cataracts in the eyes and the deformation of red blood cells that is responsible for sickle-cell anemia. Vekilov and his team of researchers have published their findings in a few recent articles, among them: "Quasi-Planar Nucleus Structure in Apoferritin Crystallization," *Nature*, **406**:494-497, 3 August, 2000; and "Control of Protein Crystal Nucleation Around the Metastable Liquid-Liquid Phase Boundary," *Proceedings of the National Academy of Sciences*, **97**:12, 6277-6281, June 6, 2000.

Additional information

OBPR: *http://spaceresearch.nasa.gov*
Microgravity research: *http://microgravity.nasa.gov*
Microgravity newsletter: *http://mgnews.msfc.nasa.gov*

Physical Sciences Division
Office of Biological and Physical Research
NASA, Code UG
Washington, DC 20546-0001

FS-2001-06-99-MSFC